JOURNAL OF BACTERIOLOGY, Nov. 2007, p. 8371–8376 Vol. 189, No. 22
0021-9193/07/$08.00+0 doi:10.1128/JB.01217-07

trans-Acting Factors and *cis* Elements Involved in Glucose Repression of Arabinan Degradation in *Bacillus subtilis*▽

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Received 28 July 2007/Accepted 30 August 2007

In *Bacillus subtilis*, the synthesis of enzymes involved in the degradation of arabinose-containing polysaccharides is subject to carbon catabolite repression (CCR). Here we show that CcpA is the major regulator of repression of the arabinases genes in the presence of glucose. CcpA acts via binding to one *cre* each in the promoter regions of the *abnA* and *xsa* genes and to two *cre*s in the *araABDLMNPQ-abfA* operon. The contributions of the coeffectors HPr and Crh to CCR differ according to growth phase. HPr dependency occurs during both exponential growth and the transitional phase, while Crh dependency is detected mainly at the transitional phase. Our results suggest that Crh synthesis may increase at the end of exponential growth and consequently contribute to this effect, together with other factors.

Bacillus subtilis secretes a vast number of polysaccharide-degrading enzymes that are able to hydrolyze plant cell wall material (30). Arabinose and xylose are the two most abundant pentoses in nature and often occur associated with hemicellulosic substrates, such as arabinans, xylans, and arabinoxylans (25, 26, 29). *B. subtilis* synthesizes enzymes, namely, endo-1,5-α-arabinanases (ABN; EC 3.2.1.99) and α-L-arabinofuranosidases (AF; EC 3.2.1.55), capable of releasing arabinosyl oligomers and arabinose from the plant tissue homoglycan arabinan (1, 13, 14, 35). In previous studies, we characterized the transcriptional regulation of three arabinan-degrading enzyme genes, *abnA* (ABN), *xsa* (AF), and *abfA* (AF), which are clustered with genes encoding enzymes that further catabolize L-arabinose (23). Expression of these genes is (i) induced by arabinose and arabinan, (ii) negatively controlled by AraR, the key regulator of the arabinose regulon, and (iii) repressed in the presence of glucose (6, 12, 23). The last phenomenon, carbon catabolite repression (CCR), is a global regulatory mechanism that ensures the selection of rapidly metabolized carbon and energy sources, such as glucose, for optimal bacterial growth (3, 30). CcpA is the master regulator of CCR in *Bacilli* and other gram-positive bacteria with low GC contents and regulates approximately 10% of the *B. subtilis* genome (2, 18). This global regulator binds to *cre*s (catabolite-responsive elements) either upstream of or in the promoter region or within coding regions of target genes (17, 34). This interaction is modulated by phosphorylation of HPr, a phosphocarrier protein of the phosphoenolpyruvate phosphotransferase system (PTS), or of Crh (an HPr-like protein) (4, 30, 33). In this work, we identified *trans*-acting factors and *cis* elements involved in glucose repression of arabinan-degrading enzyme genes. Moreover, we address the question of temporal regulation of these genes (23) by determination of the contributions of CcpA and the coeffectors HPr and Crh to glucose repression at different growth stages (exponential and postexponential [transitional] growth).

***cis*-Regulatory elements in the promoter regions of *abnA* and *xsa*.** The involvement of two *cre*s present in the *araABDLMNPQ-abfA* operon (*cre araA* and *cre araB*) (Fig. 1) in CCR was shown in previous studies (12, 17). Both *cre araA*, located between the promoter region and the *araA* gene, and *cre araB*, placed 2 kb downstream within the *araB* gene, are independently functional, and both contribute to glucose repression (12). Moreover, the level of glucose repression, 21.8-fold, measured in a strain bearing an *abfA′-lacZ* reporter fusion (IQB450) (23; see below) was only 1.6-fold higher than that observed with a strain bearing an *araAB′-lacZ* fusion, i.e., 13.4-fold (12), suggesting the absence of other *cre* active sites in glucose-mediated CCR of *abfA* expression. Expression of *abnA* and *xsa* is also subject to glucose repression (23). Potential *cre* sequences, namely, *cre abnA* (+83-TGTAAGCGCTTTCT [SubtiList position 2949135]) (Fig. 1) (17) and *cre xsa* (+1-TAAAAGCGCT TACA [SubtiList position 2914316]) (Fig. 1) (18), are present in the promoter regions of the genes. To assess the functionality of these putative regulatory sites, we introduced a single-base-pair substitution which destroyed the central symmetry in both *cre abnA* (+89 C→A) and *cre xsa* (+7 C→A) (Fig. 1). The two promoter regions, together with the respective mutated *cre*s, were fused to the *lacZ* reporter gene and introduced into the *amyE* locus. In the resulting *B. subtilis* strains, IQB472 and IQB473 (Table 1), respectively, the levels of accumulated β-galactosidase activity in the absence or presence of the inducer arabinose and under repressing conditions (arabinose plus glucose) were determined. Glucose repression was almost abolished in the mutants compared to that in strains bearing the wild-type promoters (IQB410 and IQB405) (23) (Table 2). These results indicate that both *cre abnA* and *cre xsa* are functional and that the designed mutation prevented binding of CcpA (see below) in the presence of glucose. Thus, *cre abnA* and *cre xsa* are the *cis*-acting elements involved in glucose-mediated CCR of *abnA* and *xsa* expression at the transcrip-

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▽ Published ahead of print on 7 September 2007.

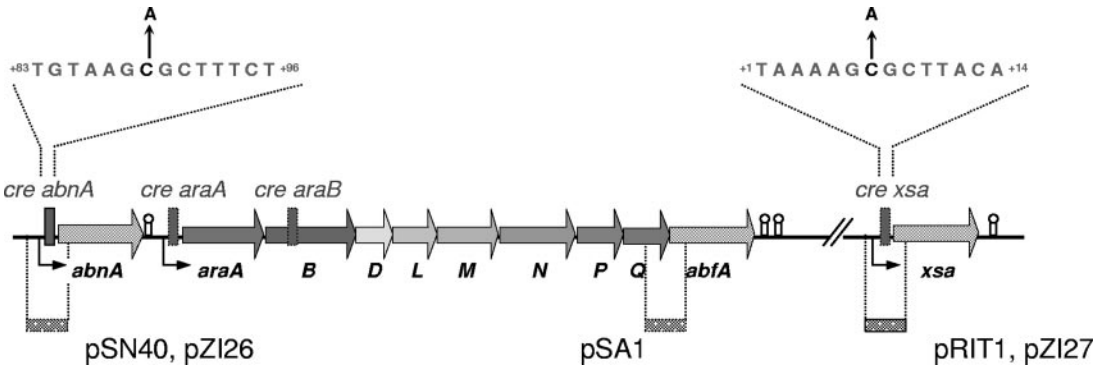

FIG. 1. Organization of the *B. subtilis* chromosome region comprising the arabinan-degrading enzyme genes *abnA*, *abfA*, and *xsa*. The three genes are represented by dotted arrows pointing in the direction of transcription. *abfA* belongs to the *araABDLMNPQ-abfA* metabolic operon, *abnA* is located immediately upstream, and *xsa* is positioned 23 kb downstream of the metabolic operon. Hairpin structures denote terminators. The dotted boxes below the physical map represent extensions of the inserts fused to the *lacZ* reporter gene in the indicated plasmids. Plasmid pSA1 was integrated into the host chromosome by means of a single-crossover (Campbell-type) recombinational event that occurred in the region of homology of the resulting strain (Table 1). Linearized DNAs from plasmids pSN40, pZI26, pZI27, and pRIT1 were used to transform *B. subtilis* strains (Table 1), and the fusions were integrated into the chromosome via double recombination at the *amyE* locus. Above the map, the DNA sequences of *cre abnA* and *cre xsa* are depicted, and the numbers indicate the positions of the *abnA* and *xsa* genes relative to the transcriptional start site. The single nucleotide change (C→A) introduced into each *cre* is shown.

tional level. In addition, the results suggest that only one *cre* in each promoter is responsible for this phenomenon.

A major role of CcpA and different contributions of effectors HPr and Crh to CCR. The arabinan-degrading enzyme genes are subject to temporal regulation, since the levels of expression of *abfA*, *xsa*, and *abnA* increase at early postexponential phase in response to arabinose and arabinan (23). Here we analyzed this effect in the presence of glucose. The expression of *abfA'-lacZ*, *xsa'-lacZ*, and *abnA'-lacZ* transcriptional fusions, integrated into the chromosomes of strains IQB450, IQB405, and IQB410, respectively (Table 1) (23), was analyzed as described above, and samples were collected 2 and 4 h after induction (t_2 and t_4, respectively), corresponding to the middle exponential and early postexponential (transitional) growth phases, respectively (Fig. 2A). Although expression of *abfA'-lacZ*, *xsa'-lacZ*, and *abnA'-lacZ* increased at t_4, the glucose repression indexes, measured as ratios between expression in the presence of arabinose and the values obtained in the presence of arabinose plus glucose, were similar during exponential growth and transitional phase (Fig. 2B, C, and D). These results suggest that the effect of glucose repression under these conditions is not affected over time.

To identify the *trans*-acting factors involved in CCR of the arabinan-degrading enzyme genes, we examined the expression of *abfA'-lacZ*, *xsa'-lacZ*, and *abnA'-lacZ* transcriptional fusions, as described above, in *B. subtilis ccpA*, *pstH1*, *crh*, *hprK*, and *araR* mutant backgrounds (Table 1). In a wild-type background, the addition of glucose caused 21.8-, 19.3-, and 6.5-fold repression of *abfA'-lacZ*, *xsa'-lacZ*, and *abnA'-lacZ* expression, respectively (strains IQB450, IQB405, and IQB410, respectively) during exponential growth phase (23). Disruption of the *ccpA* gene led to a complete loss of glucose-mediated CCR of expression from the *abfA'-lacZ* (IQB474), *xsa'-lacZ* (IQB423), and *abnA'-lacZ* (IQB422) fusions (Fig. 2), revealing its major role in glucose-mediated CCR of the arabinan-degrading enzyme genes. The *ptsH1* mutation (HPr Ser46 to Ala), which impaired HPr Ser46 phosphorylation (5), partially abolished glucose repression of *abfA'-lacZ* (IQB475), *xsa'-lacZ* (IQB467), and *abnA'-lacZ* (IQB466) fusion expression (Fig. 2). In contrast, disruption of the *crh* gene caused almost no effect on glucose repression of expression from the *abfA'-lacZ*, *xsa'-lacZ*, and *abnA'-lacZ* fusions (IQB476, IQB469, and IQB468, respectively) during exponential growth phase (t_2). Accordingly, in the *ptsH1 crh* double mutant background, the levels of glucose repression, at t_2, of *abfA'-lacZ* (IQB477), *xsa'-lacZ* (IQB471), and *abnA'-lacZ* (IQB470) fusions were only slightly lower than those observed in the single *ptsH1* mutant (Fig. 2), suggesting no or a small contribution of Crh to CCR mediated by glucose. These results are in agreement with previous observations that both CcpA and HPr, but not Crh, participate in glucose repression of the *araE* and *araAB* genes (12). However, in the *crh*-null mutant background, a significant relief of glucose repression of *abfA'-lacZ* and *xsa'-lacZ* expression, of 6.4-fold and 2.1-fold, respectively, was observed at transitional phase (t_4), suggesting a contribution of Crh to glucose-mediated CCR at late growth phases (discussed below). The disruption of the *hprK* gene, encoding the bifunctional HPr kinase/phosphorylase, which reversible phosphorylates HPr and Crh (8, 16, 24), also caused a decrease of glucose repression (in IQB478, IQB479, and IQB480) (Fig. 2).

During exponential growth of cells with an *araR*-null mutant background, we observed a twofold derepression of expression of *xsa'-lacZ* (IQB406) and *abfA'-lacZ* (IQB453) in the presence of glucose compared to that of the wild type (Fig. 2B and C). AraR is a negative regulator of the arabinose-inducible genes (6, 7, 20), and this effect was previously observed with *araE'-lacZ* and *araAB'-lacZ* fusions (12). The AraR binding sites and *cre* sequences are located close to each other in the promoter regions of the *araABDLMNPQ-abfA*, *araE*, and *xsa* genes (12, 19, 23). Since under particular physiological conditions both CcpA and AraR might be bound to the DNA in close proximity, a possible interaction (cooperation) between the two proteins in the negative control of these genes was proposed (12, 19). Interestingly, this effect was not observed

TABLE 1. *B. subtilis* strains, plasmids, and oligonucleotides used in this study

Plasmid, strain, or oligonucleotide	Relevant construction, genotype, or sequence (5′→3′)	Source or reference[a]
Plasmids		
pJM783	Integrative plasmid; promoterless *lacZ* preceded by rbs_{spoVG} and multicloning site (MCS); *cat bla*	22
pSN32	Promoterless *lacZ* preceded by rbs_{spoVG} and MCS; *cat bla*; flanked by *amyE*-5′ and *amyE*-3′	20
pMutin4	Promoterless *lacZ* preceded by rbs_{spoVG} and MCS; *lacI bla ery Pspac*	31
pRIT1	pSN32 containing a 281-bp fragment of the *xsa* promoter region in the MCS	23
pSN40	pSN32 carrying a 292-bp fragment of the *abnA* promoter region in the MCS	23
pSA1	pJM783 containing a 687-bp fragment comprising the *abfA* 5′ region in the MCS	23
pZI26	Same as pSN40, but with a single-base-pair substitution at position +89 (C→A)	This work
pZI27	Same as pRIT1, but bearing a single-base-pair substitution at position +7 (C→A)	This work
pGP211	$pBSK^+$ derivative that contains the *spc* gene inserted into the *hprK* gene	10
pZI45	Identical to pBGM6 (9), pMutin4 bearing a *crh′-lacZ* fusion	This work
pZI48	Identical to pLF2 (9), pMutin4 bearing a *ptsH′-lacZ* fusion	This work
Strains		
$168T^+$	Prototroph	12
QB5223	*trpC2 ptsH1*	15
QB7097	*trpC2 crh*::*spc*	15
WLN29	*trpC2 aroG932 ccpA*::Tn*917*	11
IQB405	*amyE*::[*xsa′-lacZ cat*]	23
IQB406	*amyE*::[*xsa′-lacZ cat*] *araR*::*km*	23
IQB410	*amyE*::[*abnA′-lacZ cat*]	23
IQB411	*amyE*::[*abnA′-lacZ cat*] *araR*::*km*	23
IQB422	*amyE*::[*abnA′-lacZ cat*] *ccpA*::Tn*917*	WLN29→IQB410
IQB423	*amyE*::[*xsa′-lacZ cat*] *ccpA*::Tn*917*	WLN29→IBQ405
IQB450	*abfA*::pSA1[*abfA′-lacZ cat*]	23
IQB453	*abfA*::pSA1[*abfA′-lacZ cat*] *araR*::*km*	23
IQB466	*amyE*::[*abnA′-lacZ cat*] *ptsH1*	pRIT1→QB5223[b]
IQB467	*amyE*::[*xsa′-lacZ cat*] *ptsH1*	pSN40→QB5223[b]
IQB468	*amyE*::[*abnA′-lacZ cat*] *crh*::*spc*	QB7097→IQB410
IQB469	*amyE*::[*xsa′-lacZ cat*] *crh*::*spc*	QB7097→IQB405
IQB470	*amyE*::[*abnA′-lacZ cat*] *ptsH1 crh*::*spc*	QB7097→IQB466
IQB471	*amyE*::[*xsa′-lacZ cat*] *ptsH1 crh*::*spc*	QB7097→IQB467
IQB472	*amyE*::[*abnA′*(+89 C→A)-*lacZ cat*]	pZI26→$168T^{+}$[b]
IQB473	*amyE*::[*xsa′*(+7 C→A)-*lacZ cat*]	pZI27→$168T^{+}$[b]
IQB474	*abfA*::pSA1[*abfA′-lacZ cat*] *ccpA*::Tn*917*	WLN29→IBQ450
IQB475	*abfA*::pSA1[*abfA′-lacZ cat*] *ptsH1*	pSA1→QB5223
IQB476	*abfA*::pSA1[*abfA′-lacZ cat*] *crh*::*spc*	QB7097→IQB450
IQB477	*abfA*::pSA1[*abfA′-lacZ cat*] *ptsH1 crh*::*spc*	QB7097→IQB476
IQB478	*abfA*::pSA1[*abfA′-lacZ cat*] *hprK*::*spc*	pGP211→IQB450[b]
IQB479	*amyE*::[*abnA′-lacZ cat*] *hprK*::*spc*	pGP211→IQB410[b]
IQB480	*amyE*::[*xsa′-lacZ cat*] *hprK*::*spc*	pGP211→IQB405[b]
IQB495	*crh*::pZI45[*crh′-lacZ erm* (::pMutin4)]	pZI45→$168T^{+}$
IQB496	*ptsH*::pZI48[*ptsH′-lacZ erm* (::pMutin4)]	pZI48→$168T^{+}$
Oligonucleotides[c]		
ARA204	−8-GGTTACTTTAAAAG<u>A</u>GCTTACATTCATGC-+21 (*xsa*)	
ARA205	GCATGAATGTAAGC<u>T</u>CTTTTAAAGTAACC	
ARA206	+66-GGATAAATAATCTAATTTGTAAG<u>A</u>GCTTTCTAAAATAAAGG-+106 (*abnA*)	
ARA207	CCTTTATTTTAGAAAGC<u>T</u>CTTACAAATTAGATTATTTATCC	

[a] Arrows indicate transformation, performed as previously described (27), and point from donor DNA to the recipient strain.
[b] Transformation was carried out with linearized plasmid DNA.
[c] Substituted residues in oligonucleotides are underlined. The position relative to the transcriptional start site of the *xsa* and *abnA* genes is indicated in the forward primer. Site-directed mutagenesis was performed using a QuikChange kit (Stratagene).

for the *abnA′-lacZ* fusion in an *araR*-null mutant background (strain IQB411) (Fig. 2D; Table 1). This observation is in agreement with a different localization of *cre abnA* relative to the AraR binding site in the *abnA* promoter and also to a weak control of *abnA* expression by AraR as a result of a distinct mechanism of action (23).

Crh expression increases at transitional growth phase. The contribution of both effectors HPr and Crh to CCR by glucose can depend on the growth conditions, as shown for the *hut* operon, which responds to HPr in rich LB medium and to both HPr and Crh in minimal medium (36). Crh is the major effector for CCR of the *citM* gene in a medium containing succinate and glutamate (32). In connection with this, it was recently shown that the nature of the carbon source influences *ptsH* and *crh* expression (9). Using *ptsH′-lacZ* and *crh′-lacZ* transcription and translational fusion analysis, it was suggested that the different contributions of Crh and HPr to CCR might be due to the drastic differences in their synthesis rates under the conditions that cause CCR. In the presence of PTS carbohydrates, such as glucose, the synthesis level of HPr during exponential

TABLE 2. Single-base-pair substitutions in the *cre*s located in the promoter regions of the *abnA* and *xsa* genes[a]

Promoter fusion and strain (relevant genotype)	β-Galactosidase activity (Miller units)[b]			Glucose repression index[c]
	−Ara	+Ara	+Ara +Glc	
abnA'-lacZ				
IQB410[d] (WT)	3.2 ± 0.7	10.0 ± 0.8	1.6 ± 0.3	6.5
IQB472 (*cre abnA*$_{mut}$)	9.1 ± 1.3	80.7 ± 11.4	41.3 ± 3.3	1.9
xsa'-lacZ				
IQB405[d] (WT)	7.5 ± 0.2	117.3 ± 3.9	9.2 ± 0.5	19.3
IQB473 (*cre xsa*$_{mut}$)	4.0 ± 0.5	163.1 ± 12.6	76.8 ± 9.5	2.1

[a] Strains containing different promoter-*lacZ* fusions were grown on C minimal medium (21) supplemented with 1% (wt/vol) casein hydrolysate in the absence of sugar (−Ara), in the presence of 0.4% (wt/vol) arabinose (+Ara), and in the presence of 0.4% (wt/vol) arabinose plus 0.4% (wt/vol) glucose (+Ara +Glc). Antibiotics used as selective markers were added as appropriate. Samples were analyzed 2 h after the addition of sugar, as previously described (27).

[b] The levels of accumulated β-galactosidase activity, determined as previously described (27), represent the averages ± standard deviations of two measurements each from three independent experiments.

[c] The glucose repression index was calculated as the ratio of the level of expression (in Miller units) obtained in the presence of arabinose to the value determined in the presence of glucose.

[d] Data are from the work of Raposo et al. (23).

growth is 100-fold higher than that of Crh, which may explain the minor role of the latter in certain circumstances (9). Moreover, CcpA binds Crh(Ser-P) more weakly than HPr(Ser-P) (28). We have previously shown the absence of a Crh contribution to glucose repression of the *araABDLMNPQ-abfA* and *araE* genes in both C minimal medium supplemented with casein hydrolysate and CSK minimal medium (12). To further investigate the lack of Crh dependency during exponential growth and its possible contribution to glucose repression of the arabinan-degrading enzyme genes during the transitional growth phase, we analyzed the expression of both *ptsH'-lacZ* and *crh'-lacZ* transcriptional fusions at different growth stages. The constructed transcriptional fusions are identical to those described by Görke et al. (9). In *B. subtilis* strains bearing the fusions, the complete *ptsH* and *crh* coding regions are restored downstream from the *lacZ* insertion cassette. Their expression is directed by the IPTG (isopropyl-β-D-thiogalactopyranoside)-inducible *Pspac* promoter in order to prevent polar effects caused by insertions into their natural chromosomal loci (Table 1). Expression from the *crh'-lacZ* (IQB495) (Table 1)

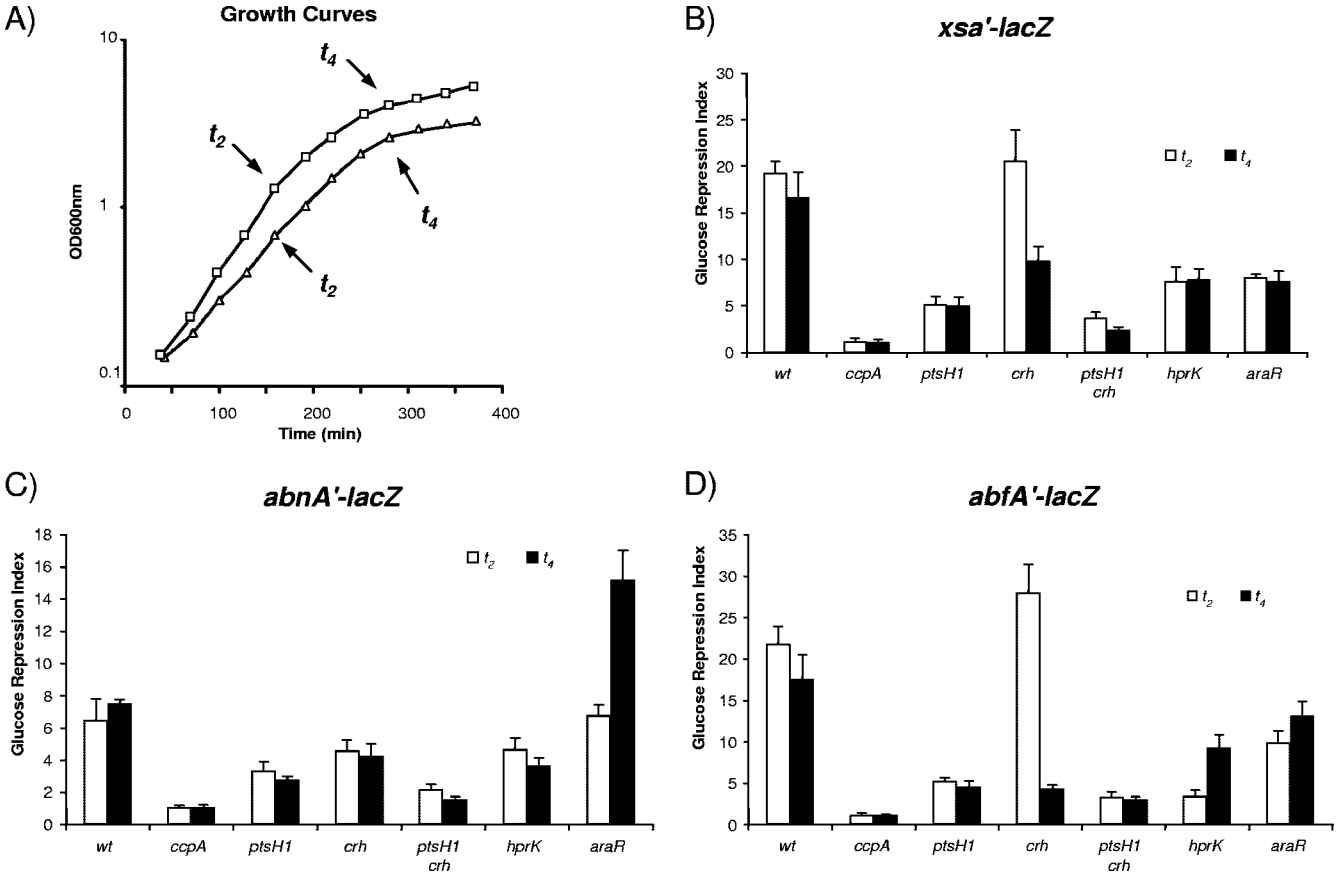

FIG. 2. Effects of *ccpA*, *ptsH1*, *crh*, *hprK*, and *araR* mutations on catabolite repression of the *xsa*, *abnA*, and *abfA* genes. (A) Growth curves for wild-type (squares) and *ccpA*-null mutant (triangles) *B. subtilis* strains in C minimal medium (21) supplemented with 1% (wt/vol) casein hydrolysate in the presence of 0.4% (wt/vol) arabinose plus 0.4% (wt/vol) glucose. Exponential growth phase (t_2) and transitional phase (t_4) are indicated. The strains bearing the *xsa'-lacZ* (B), *abnA'-lacZ* (C), and *abfA'-lacZ* (D) transcriptional fusions were grown in the absence of sugar, in the presence of 0.4% (wt/vol) arabinose, and in the presence of 0.4% (wt/vol) arabinose plus 0.4% (wt/vol) glucose. The levels of accumulated β-galactosidase activity, expressed as Miller units, were measured at hours 2 (t_2) and 4 (t_4) after induction (addition of arabinose). The results are represented by the glucose repression index, calculated as the ratio between the level of accumulated Miller units obtained in the presence of arabinose and the value determined in the presence of arabinose plus glucose. Each value is the average of two measurements each from three independent experiments.

TABLE 3. Expression from *crh′-lacZ* and *ptsH′-lacZ* fusions in minimal media at different growth stages[a]

Medium	Strain (promoter fusion)	Time[b]	β-Galactosidase activity (Miller units)[c]			
			−Ara	+Ara	+Ara +Glc	+Glc
C	IQB495 (*crh′-lacZ*)	t_2	36.0 ± 3.8	31.5 ± 2.4	25.6 ± 1.2	23.9 ± 2.4
		t_4	37.0 ± 0.8	37.8 ± 1.2	56.8 ± 3.2	56.9 ± 2.6
	IQB496 (*ptsH′-lacZ*)	t_2	97.4 ± 14.9	94.9 ± 10.1	315.4 ± 18.4	322.7 ± 16.2
		t_4	87.5 ± 7.1	94.3 ± 13.3	323.2 ± 16.3	322.7 ± 47.2
CSK	IQB495 (*crh′-lacZ*)	t_2	31.4 ± 3.4	28.4 ± 3.5	24.5 ± 3.7	29.1 ± 2.3
		t_4	28.3 ± 1.3	27.1 ± 3.2	43.7 ± 6.8	45.5 ± 2.7
	IQB496 (*ptsH′-lacZ*)	t_2	59.7 ± 5.4	56.7 ± 6.0	204.0 ± 31.1	229.1 ± 16.2
		t_4	55.0 ± 5.9	46.5 ± 8.3	195.9 ± 10.3	228.5 ± 38.8

[a] Strains containing different promoter-*lacZ* fusions were grown on C minimal medium (21) supplemented with casein hydrolysate or in CSK minimal medium (9) with IPTG (1 mM) in the absence of sugar (−Ara), in the presence of arabinose (+Ara), in the presence of arabinose plus glucose (+Ara +Glc), and in the presence of glucose (+Glc). Antibiotics used as selective markers were added as appropriate.

[b] Samples were analyzed 2 h (t_2) and 4 h (t_4) after the addition of sugars, which correspond to exponential growth phase and transitional phase, respectively.

[c] The levels of accumulated β-galactosidase activity represent the averages of two measurements each from three independent experiments.

and *ptsH′-lacZ* (IQB496) (Table 1) transcriptional fusions during exponential growth phase (t_2) was comparable in both C minimal medium supplemented with casein hydrolysate and CSK minimal medium (Table 3). The presence of arabinose, a non-PTS sugar, did not influence *crh* and *ptsH* expression (Table 3). In addition, the β-galactosidase activities obtained for *crh′-lacZ* and *ptsH′-lacZ* in CSK minimal medium plus glucose (29 and 229, respectively) (Table 3) were very similar to those obtained by Görke et al. (9). Interestingly, at transitional growth phase (t_4), the expression of *crh* increased about twofold in the presence of glucose and in both media. In contrast, *ptsH* expression was unaffected by the growth phase. These results suggest that increased expression of *crh* at the transcriptional level during transitional phase in the presence of glucose may lead to a higher cellular concentration of Crh than of HPr. Thus, this difference in synthesis level may contribute to the glucose repression relief effect on *abfA′-lacZ* and *xsa′-lacZ* expression in the *crh*-null mutant background (Fig. 2). However, due to a still expected higher concentration of HPr than Crh under these conditions, other factors, such as intracellular variation of certain metabolites which may influence Crh(Ser-P) phosphorylation and/or CcpA-Crh(Ser-P) complex formation, are likely to contribute to Crh(Ser-P)-mediated glucose repression at transitional phase.

Concluding remarks. CCR of the arabinan-degrading enzyme genes is mediated by binding of CcpA to one *cre* located downstream near the promoter regions of *abnA* and *xsa* and to two *cre*s present in the *araABDLMNPQ-abfA* operon, with *cre araA* located near the promoter and *cre araB* positioned within the *araB* gene. Our data suggest that during exponential growth phase, glucose-mediated CCR is mediated by CcpA associated with the coeffector HPr(Ser-P), and that towards the end of exponential growth, at transitional phase, glucose repression is achieved by both CcpA-HPr(Ser-P) and CcpA-Crh(Ser-P) complexes.

We thank Jörg Stülke for the kind gift of plasmid pGP211.

This work was partially supported by grants POCTI/AGR/36212/00 and POCI/AGR/60236/2004 from Fundação para a Ciência e Tecnologia (FCT) and FEDER to I.D.S.-N. and by fellowship SFRH/BD/18238/2004 from FCT to J.M.I.

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